PATENT COOPERATION TREAT

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *day/month/year*	9 DEC 2004
Applicant's or agent's file reference 12177502/VPA/JRC	IMPORTANT NOTIFICATION

International Application No.	International Filing Date	Priority Date
PCT/AU2003/001021	12 August 2003	12 August 2002

Applicant

THE UNIVERSITY OF QUEENSLAND et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU	Authorized officer
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12177502/VPA/JRC	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/001021	International Filing Date *(day/month/year)* 12 August 2003	Priority Date *(day/month/year)* 12 August 2002
International Patent Classification (IPC) or national classification and IPC Int. Cl. [7] C12N 5/16 A61K 35/14		
Applicant THE UNIVERSITY OF QUEENSLAND et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [X] Certain defects in the international application
   - VIII [X] Certain observations on the international application

Date of submission of the demand 3 March 2004	Date of completion of the report 6 December 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer LEXIE PRESS Telephone No. (02) 6283 2677

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001021

I. Basis of the report

1. With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1 to 65**, as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **68 to 73**, received on **10 November 2004** with the letter of **10 November 2004**
- [X] the drawings, pages **1/25 to 25/25**, as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

International application No.
PCT/AU2003/001021

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-58	YES
	Claims -	NO
Inventive step (IS)	Claims 1-58	YES
	Claims -	NO
Industrial applicability (IA)	Claims 1-58	YES
	Claims -	NO

2. Citations and explanations (Rule 70.7)

The present invention relates to methods of producing antigen presenting cells (APCs), such as dendritic cells (DCs) and macrophages, that express CD40 activity at a reduced level or functional activity, and the use of the APCs in suppressing immune responses. Methods for generating APCs of the desired phenotype comprise contacting APCs or their precursors with inhibitors of NF-κB or CD40.

This report is based on the amended claims received on 10 November 2004.

Documents D1 to D8 were identified in the International Search Report and their relevance to the novelty and inventive step of the present application was discussed in the first opinion. As a consequence of amendment of the claims, D1 to D6 are no longer considered relevant. The claims are entitled to the claimed priority and therefore D7 and D8 are not considered as part of the prior art base for the assessment of novelty and inventive step.

D1 McRae et al (2000) Blood Vol 96(1): 210-217

D2 Yoshimura et al. (2001) International Immunology. Vol 13(5): 675-683

D3 Giannoukakis et al. (2000) Molecular Therapy. Vol 1(5): 430-437

D4 Verhasselt (1999) J. Immunol. Vol 162(5): 2569-2574

D5 Pan et al. (2001) Immunology Letters Vol 76: 153-16

D6 Ruby et al. (2002) Molecular Pharmacology. Vol 62 (3): 722-728

D7 O'Sullivan and Thomas (2002) J Immunol. Vol 168(11): 5491-5498

D8 Caldwell et al (2003) J Immunol. Vol 171(4): 1676-83

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

The numbering of the claims pages submitted with the letter of 10 November 2004, is not continuous with the numbering of the description pages.

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VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-17, 35-40, and 46-58 appear not be supported by the description. The specification discloses only one means for producing APCs with reduced expression of CD40. The disclosed method involves inhibiting NF-κB activity in precursors of APCs. In contrast the claims are drawn to APCs with reduced levels of CD40 expression *per se* and their applications, rather than APCs prepared by inhibiting NF-κB. Without limitation of the claims to the disclosed method for achieving APCs of the desired phenotype, the claims embody subject matter which is not supported by the description.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V

Novelty and Inventive Step

D1 discloses that monocyte derived DCs developed in the presence of IFN-β, express reduced levels of CD40 relative to DCs cultured in the absence of IFN-β. The cells have features of mature DCs, including high CD86 and MHC class II. The IFN-β treated DCs have reduced capacity to support T-cell proliferation through allostimulation.

D2 discloses that the authors over-expressed the NF-κB inhibitor, IκBα, in mature DC and demonstrated that DC antigen presentation is NF-κB dependent. Blocking NF-κB down regulates expression of costimulatory molecules CD40 and CD86. The investigators substantiated their conclusion regarding the role of NF-κB in antigen presentation by using proteosome inhibitor I instead of over-expressing IκBα.The authors suggest that NF-κB is a suitable target for modulating DC antigen presentation and inhibiting T cell dependent immune responses.

D3 discloses that treatment of DC precursors with ds oligodeoxyRNAs containing binding sites for NF-κB suppressed ıe cell surface expression of CD40 and CD86 and the expression of these molecules could not be augmented by activation of the DCs with LPS. The authors also used these APCs to promote tolerance in heart transplant patients.

D4 discloses that NAC inhibits the activity of NF-κB in DC and down regulates expression of CD40 and CD86 upon activation with LPS.

D5 teaches that dexamethasone down regulates CD40 and CD86 and impairs DC ability to activate T cells.

D6 discloses the concept that NF-κB activation is necessary for T-cell activation by DC. There is no disclosure of cells with reduced expression of CD40.

In summary, none of the documents teach APCs that produce CD40 at a level or functional activity that is less than 50% of that produced by an activated DC, or that such APCs can be generated by inhibiting NF-κB in immature monocyte-derived DC precursors. Therefore D1 to D6 are not prejudicial to the novelty or inventiveness of any of claims 1 to 58.

WHAT IS CLAIMED IS:

1. An isolated antigen-presenting cell for modulating an immune response, which is characterised by producing CD40, or its equivalent, at a level or functional activity that is less than about 50% of that produced by an activated dendritic cell.

2. An antigen-presenting cell according to claim 1, wherein CD40, or its equivalent, is produced at a level or functional activity that is less than about 1% of that produced by an activated dendritic cell.

3. An antigen-presenting cell according to claim 1, which cannot be induced to express CD40, or its equivalent, at an equivalent level and/or functional activity as that produced by an activated antigen presenting cell.

4. An antigen-presenting cell according to claim 1, wherein CD40, or its equivalent, is produced at a level or functional activity that is lower than that produced by an immature dendritic cell.

5. An antigen-presenting cell according to claim 1, which cannot be induced to express CD40, or its equivalent, at a higher level and/or functional activity than that produced by an immature antigen-presenting cell.

6. An antigen-presenting cell according to claim 1, which is other than a B lymphocyte.

7. An antigen-presenting cell according to claim 1, which is selected from monocytes, macrophages, cells of myeloid lineage, dendritic cells or Langerhans cells.

8. An antigen-presenting cell according to claim 1, which is a dendritic cell.

9. An antigen-presenting cell according to claim 1, which is a macrophage.

10. An antigen-presenting cell according to claim 1, which produces NF-κB or a component thereof, at a level or functional activity which is lower than that produced by a mature or activated dendritic cell.

11. An antigen-presenting cell according to claim 1, which cannot be induced to express NF-κB or component thereof, at a higher level and/or functional activity than an immature antigen presenting cell.

12. An antigen-presenting cell according to claim 1, which produces NF-κB or a component thereof, at a level or functional activity that is lower than that produced by an immature dendritic cell.

13. An antigen-presenting cell according to any one of claim 10 to 12, wherein the component is RelB.

14. An antigen-presenting cell according to claim 1, which produces an immunostimulatory molecule.

15. An antigen-presenting cell according to claim 14, wherein the immunostimulatory molecule comprises CD86 or its equivalent.

16. An antigen-presenting cell according to claim 14, wherein the immunostimulatory molecule is produced at a level or functional activity which is at least about 10% of that produced by an activated dendritic cell.

17. An antigen-presenting cell according to claim 14, wherein the immunostimulatory molecule is produced at a level or functional activity which is the same as that produced by an activated dendritic cell.

18. An antigen-presenting cell according to claim 1, which is produced by a process comprising contacting a precursor of the antigen-presenting cell with an NF-κB inhibitor for a time and under conditions sufficient to differentiate an antigen-presenting cell from the precursor and to inhibit or otherwise reduce the level and/or functional activity of NF-κB in the cell.

19. An antigen-presenting cell according to claim 18, wherein the precursor is derived from monocytes or bone marrow.

20. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor inhibits nuclear translocation of NF-κB, or a component thereof.

21. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor inhibits nuclear translocation of RelB.

22. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an antisense nucleic acid molecule or oligonucleotide, which is complementary or encodes at least a portion of a NF-κB subunit selected from p50, p65 or RelB.

23. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an inhibitor of RelB or p50.

24. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an inhibitor of RelB or p50, which is selected from a ribozyme that selectively destroys RNA encoding NF-κB or component thereof, an antisense molecule which prevents transcription of NF-κB or component thereof, or an antigen-binding molecule that blocks NF-κB action.

25. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an indirect inhibitor of NF-κB selected from inhibitors of IκB degradation, inhibitors of IκB phosphorylation, inhibitors of IκB ubiquitination and inhibitors of proteolytic degradation of IκB.

26. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an inhibitor of IκB phosphorylation.

27. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is BAY 11-7082.

28. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an indirect inhibitor of NF-κB selected from inhibitors of proteolysis and inhibitors of nuclear translocation of NF-κB.

29. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an inhibitor of nuclear translocation of NF-κB selected from deoxyspergualin or deoxyspergualin derivatives or analogues.

30. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an inhibitor of proteolysis selected from proteosome inhibitors.

31. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is a proteosome inhibitor selected from PSI, ALLN, lactacystin, MG-132, C-LFF and calpain inhibitors.

32. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is an indirect inhibitor of NF-κB selected from caffeic acid phenethyl ester, pyrrolidine dithiocarbonate, lovastatin, aselastine HCL, tepaxalin, (-)-epi gallocatechin-3-gallate, phenyl-N-tert-butylnitrone, quercetin, cucumin or E330.

33. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor inhibits proteolysis.

34. An antigen-presenting cell according to claim 18, wherein the NF-κB inhibitor is a proteosome inhibitor.

35. An antigen-presenting cell according to claim 1, which is produced by a process comprising contacting an antigen-presenting cell, or its precursor, with an inhibitor of CD40, or its equivalent, for a time and under conditions sufficient to produce a modified antigen-presenting cell that produces CD40, or its equivalent, at a reduced or abrogated level or functional activity relative to that of the antigen-presenting cell or its precursor.

36. An antigen-presenting cell according to claim 35, wherein the process further comprises contacting the antigen-presenting cell, or its precursor, or the modified antigen-presenting cell, with an agent that increases the level or functional activity of an immunostimulatory molecule for a time and under conditions sufficient to enhance or otherwise elevate the level or functional activity of the immunostimulatory molecule in the antigen-presenting cell, or its precursor, or the modified antigen-presenting cell.

37. An antigen-presenting cell according to claim 35, wherein the process further comprises contacting the antigen-presenting cell, or its precursor, or the modified antigen-presenting cell, with an agent that increases the level or functional activity of CD86 or its equivalent, for a time and under conditions sufficient to enhance or otherwise elevate the level or functional activity of CD86 or its equivalent in the antigen-presenting cell, or its precursor, or the modified antigen-presenting cell.

38. A method of producing antigen-presenting cells for modulating an immune response to a target antigen, comprising contacting an antigen-presenting cell, or its precursor, with an antigen that corresponds to the target antigen, or with a polynucleotide from which the antigen is expressible, for a time and under conditions sufficient for the antigen or a processed form thereof to be presented by the antigen-presenting cell, or its precursor, wherein antigen-presenting cell, or its precursor, is characterised by producing CD40, or its equivalent, at a level or functional activity that is less than about 50% of that produced by an activated dendritic cell.

39. A method according to claim 38, wherein the antigen presentation is restricted by major histocompatability (MHC) molecules.

40. An antigen-specific antigen-presenting cell for modulating an immune response to a target antigen, which is produced by contacting an antigen-presenting cell, or its precursor, with an antigen that corresponds to the target antigen, or with a polynucleotide from which the antigen is expressible, for a time and under conditions sufficient for the antigen or a processed form thereof to be presented by the antigen-presenting cell, or its precursor, wherein antigen-presenting cell, or its precursor, is characterised by producing CD40, or its equivalent, at a level or functional activity that is less than about 50% of that produced by an activated dendritic cell.

41. A method of producing antigen-presenting cells for modulating an immune response to a target antigen, comprising contacting a precursor of the antigen-presenting cell with an NF-κB inhibitor and with an antigen that corresponds to the target antigen, or with a polynucleotide from which the antigen is expressible, for a time and under conditions sufficient to differentiate an antigen-presenting cell from the precursor and to inhibit or otherwise reduce the level or functional activity of NF-κB in the cell, wherein the antigen or a processed form thereof is presented by the antigen-presenting cell so produced.

42. A method according to claim 41, wherein the immune response is mediated by T lymphocytes.

43. A method according to claim 41, wherein the T lymphocytes are selected from cytotoxic T lymphocytes (CTLs) and T helper lymphocytes.

44. A method according to claim 41, wherein the antigen is selected from a protein antigen, a particulate antigen, an alloantigen, an autoantigen, an allergen, a bacterial antigen, a viral antigen or a parasitic antigen or immune complex.

45. A method according to claim 41, wherein the modulation of the immune response is selected from inducing a tolerogenic response, or the suppression of a future or existing immune response, to a specified antigen or group of antigens.

46. A method for producing T lymphocytes that exhibit anergy for a target antigen, comprising contacting a population of T lymphocytes, or their precursors, with an antigen-specific antigen-presenting cell, which is characterised by producing CD40, or its equivalent, at a level or functional activity that is less than about 50% of that produced by an activated dendritic cell, for a time and under conditions sufficient to produce the anergic T lymphocytes.

47. A method according to claim 46, wherein the T lymphocytes are selected from cytotoxic T lymphocytes (CTLs) and T helper lymphocytes.

48. A method according to claim 46, wherein the antigen is selected from a protein antigen, a particulate antigen, an alloantigen, an autoantigen, an allergen, a bacterial antigen, a viral antigen or a parasitic antigen or immune complex.

49. A T lymphocyte that exhibits anergy for a target antigen, which is produced by contacting a T lymphocyte, or its precursor, with an antigen-specific antigen-presenting cell, which is characterised by producing CD40, or its equivalent, at a level or functional activity that is less than about 50% of that produced by an activated dendritic cell, for a time and under conditions sufficient to produce the anergic T lymphocyte.

50. A method for modulating the immune response to an antigen, comprising administering to a patient in need of such treatment an antigen-specific antigen-presenting cell for a time and under conditions sufficient to modulate the immune response, wherein the antigen-specific antigen-presenting cell is produced by contacting an antigen-presenting cell with an antigen that corresponds to the target antigen, or with a polynucleotide from which the antigen is expressible, for a time and under conditions sufficient for the antigen or a processed form thereof to be presented by the antigen-presenting cell, wherein antigen-presenting cell is characterised by producing CD40, or its equivalent, at a level or functional activity that is less than about 50% of that produced by an activated dendritic cell.

51. A method for modulating the immune response to an antigen, comprising administering to a patient in need of such treatment an anergic T lymphocyte for a time and under conditions sufficient to modulate the immune response, wherein the anergic T lymphocyte is produced by contacting a population of T lymphocytes, or their precursors, with an antigen-specific antigen-presenting cell, which is characterised by producing CD40, or its equivalent, at a level or functional

activity that is less than about 50% of that produced by an activated dendritic cell, for a time and under conditions sufficient to produce the anergic T lymphocytes.

52. Use of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 for inducing an anergic response.

53. Use of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 for treating or preventing an allergy.

54. Use of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 for treating or preventing an autoimmune disease.

55. Use of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 for treating or preventing transplant rejection in a patient.

56. A method for treatment and/or prophylaxis of a disease or condition whose symptoms or aetiology are associated with the presence of an immune response, comprising administering to a patient in need of such treatment or prophylaxis an effective amount of one or both of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 .

57. Use of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 in the preparation of a medicament for the modulation of an immune response.

58. Use of an antigen-specific antigen-presenting cell according to claim 40 and/or of an anergic T lymphocyte according to claim 49 in the study and modulation of immune responses.